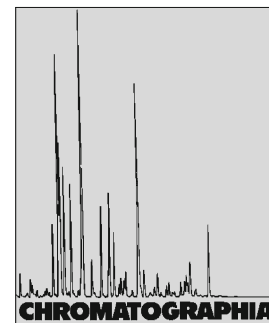


# Establishment of the Model CD40 Cell Membrane Chromatography and Its Chromatographic Characteristics



2008, 67, 829–831

Guangde Yang, Rong Lin<sup>✉</sup>, Zhen Hu, Jiye Zhang, Chunjie Han, Langchong He, Weirong Wang

School of Medicine, Xi'an Jiaotong University, Shaanxi 710061, China; E-Mail: linrong@mail.xjtu.edu.cn

Received: 10 July 2007 / Revised: 5 December 2007 / Accepted: 23 January 2008  
Online publication: 24 April 2008

## Abstract

A new model of atherosclerosis cell membrane chromatography has been established by using a CD40 cell membrane stationary phase (CD40 CMSP) prepared by immobilizing the CD40 cell membrane onto the surface of a silica carrier. The surface and chromatographic characteristics of CD40 CMSP were studied. The retention characteristics of anti-CD40 antibody and statins (lovastatin, simvastatin and pravastatin) were also investigated using this model. Affinities of the anti-CD40 antibody and statins toward CD40 cell membrane and receptors were based on the determination of  $\log k'$  values (the logarithm of capacity factor of a solute). There was a significant correlation between the affinity in the CD40–CMC and the effect in vitro for the pharmacological effect.

## Keywords

Cell membrane chromatography  
CD40  
Atherosclerosis

## Introduction

CD40 is a 50 kDa integral membrane protein of the tumor necrosis factor (TNF) receptor family. Expression of CD40 has been documented for a variety of non-immune cells including endothelial cells, fibroblasts and vascular smooth muscle cells. CD40L (39 kDa) serves as the ligand for CD40 and is also a membrane-bound member of the TNF gene family that is expressed by T-cells and

activated platelets [1]. The CD40–CD40L system is proven to be an important mediator of several autoimmune and chronic inflammation diseases [2]. Interruption of CD40–CD40L signaling not only reduces the initiation and progression of atherosclerotic lesions in vivo [3, 4] but also impacts on plaque architecture [5, 6]. Moreover, it has been reported that CD40–CD40L serves as a key regulator of this process which may render this therapeutically useful for the

treatment of atherosclerotic conditions [4].

The purpose of the present work was to develop a new model of CD40 cell membrane chromatography (CD40–CMC) using anti-CD40 antibody and statin derivatives (lovastatin, simvastatin and pravastatin) as model molecules.

## Experimental

### Instruments

The chromatographic system consisted of a LC-10ATvp chromatographic pump, a SPD-10Avp detector (Shimadzu, Japan), a 7725i hand sampling valve (Rheodyne, USA), and a LC-Solution chromatographic work station (Shimadzu, Japan).

### Materials and Reagents

Macroporous silica (3–5  $\mu\text{m}$ , 100 Å) (Institute of Chemistry of the Chinese Academy of Sciences, China) were used. An endothelial cell line derived from human umbilical vein endothelial cells (HUVEC) was purchased from the Shanghai Institute of Cell Biology in the Chinese Academy of Sciences (Shanghai, China). Fetal bovine serum was purchased from Hangzhou Sijiqing Serum Works. RPMI1640 and trypsin were

**Table 1.** Retention characteristics in CD40–CMC system

Treatment	Pure silica log $k'$	CD40 CMSP log $k'$
Anti-CD40 antibody	0.20	1.68
Lovastatin	0.13	1.56
Simvastatin	0.23	1.32
Pravastatin	0.37	1.11

purchased from Sigma (USA). Lovastatin, simvastatin and pravastatin were purchased from Sigma (St Louis, MO, USA). Anti-CD40 antibodies were purchased from Santa Cruz Biotechnology (St Cruz, CA, USA). Dibasic sodium phosphate, sodium chloride, potassium chloride, hydrochloric acid and phosphoric acid were of analytical grade. Deionized water was of HPLC grade. *Salvia miltiorrhiza* was identified by the Pharmacognostical Lab from the College of Pharmacy, Xi'an Jiaotong University.

## Establishment of the Model of CD40 CMC

### Cell Culture

HUVEC overexpressing CD40 was cultured in monolayer in RPMI1640 supplemented with 10% fetal bovine serum and propagated for several days in a humidified incubator at 37 °C and 5% CO<sub>2</sub> [7, 8]. Native HUVEC was cultured under similar conditions as a minus control. The extent of CD40 protein expression in both HUVEC and native HUVEC was determined by Becton Dickinson FACScan flow cytometry using CellQuest software (Becton Dickinson).

### Preparation and Chromatographic Characteristics of CD40 CMSP

After HUVEC high throughput expression, CD40 cells were cultured until 80% confluent, digested with 0.25% trypsin and centrifuged at 1,000g for 10 min. The precipitate was resuspended in deionized water and left for 30 min, sonicated for 10 min and centrifuged at 2,000g for 20 min. The supernatant was centrifuged at 12,000g for 20 min. All procedures were performed at 4 °C [9]. The protein level, K<sup>+</sup>–Na<sup>+</sup>–ATP enzy-

matic activity of the cell membrane have also been determined. The membrane protein level was 2.14 mg mL<sup>-1</sup>. The CD40 cell membranes were stored at –34 °C.

Three hundred mg activated silica was added to a 10 mL reaction tube and then the CD40 cell membrane suspension was slowly added under the evacuation condition at 4 °C. The adsorption of the cell membranes on the activated silica surface was carried out for 1 h until equilibrium. Then, the supernatant in the tube was removed by centrifugation and the CD40 CMSP was washed with Tris–HCl buffer until there was no residual free cell membrane present.

The surface characteristics of CD40 CMSP were analyzed by a scanning electron microscope and a surface energy spectrometer.

### Chromatographic Conditions

The CD40 CMSP was dispersed in a buffer and packed as a slurry under low pressure (30 mm × 2 mm column). The flow rate of the mobile phase (50 mmol L<sup>-1</sup> phosphate buffer solution at pH 7.4) was set to 0.5 mL min<sup>-1</sup> at 37 °C. The detection wavelength was 254 nm. Before injection the chromatographic system required around 3–4 h in order to establish equilibrium. Five microliters anti-CD40 antibodies and statins (lovastatin, simvastatin and pravastatin) were injected separately.

## Results and Discussion

### Expression of CD40 in Transfected HUVEC

The expression of CD40 protein in the transfected HUVEC and native HUVEC was detected by Becton Dickinson FACScan flow cytometry using Cell-

Quest software (Becton Dickinson). The result showed that both transfected HUVEC and native HUVEC expressed CD40 protein, and the expression of CD40 in the transfected HUVEC was 91.2 ± 8.7%, which was much higher than that in native HUVEC (23.6 ± 2.6%). The result indicated that the transfected HUVEC highly expresses CD40 protein, which is applied to improvement of the specificity and selectivity of CMC model.

### The Surface Characteristic of CD40 CMSP

The surface energy spectrum of the silica carrier revealed the presence of a low peak of oxygen and a high peak of silica. In case of the CD40 CMSP, not only a new carbon peak was detected but also the silica peak almost disappeared. This indicated that the surface of silica carrier was completely covered by the cell membranes.

### Retention Behavior of Reagents in CD40–CMC

The chromatographic column packed with the CD40 CMSP did not only maintain the enzymatic activity but also showed bio-affinity. The column system was able to selectively recognize the drug molecule or ingredient acting on the CD40 cell membrane. As is the case with other chromatographic methods, the affinity of the solute can be expressed by using the log capacity factor (log  $k'$ ):

$$\log k' = \log [(t_R - t_0)/t_0]$$

where  $t_R$  is the retention time of solute,  $t_0$  the elution time of a non-retentive solvent.

Table 1 shows a comparison of retention characteristics between anti-CD40 antibodies and a selection of statins (lovastatin, simvastatin and pravastatin). It can be seen that chromatographic retention was observed when the CD40 CMSP system was used but not when only pure silica was present which indicated antibody–receptor affinity.

## Correlation with Pharmacological Activity

The CD40 expression in the transfected HUVEC was  $91.2 \pm 8.7\%$  without drug interfere. Flow cytometry analysis revealed that the presence of antibodies and three statins reduced CD40 expression in the transfected HUVEC cell line after a 24 h preincubation period. Inhibition occurred in the following order: antibody (63%) > lovastatin (54%) > simvastatin (43%) > pravastatin (28%). This order had a very close correlation with the log  $k'$  value obtained from the CD40-CMC system ( $r^2 = 0.9954$ ).

The results indicated that there was a significant correlation between the affinity in the CD40-CMC and the effect in vitro for the pharmacological effect. The stable highly expression of CD40 CMC was prepared to screen the effective anti-atherosclerotic components of *Salvia miltiorrhiza* [10].

## Conclusions

The model of CD40 cell membrane chromatography was established using

cell membrane of CD40 cell cultured in vitro.

The in vivo interaction between statins and the CD40 cell membrane receptor can be quantitatively assessed by using the CD40 CMC system in vitro. The log  $k'$  affinity parameter obtained by the system showed a significant correlation with drug effects.

## Acknowledgments

This work was supported by a grant from the National Natural Science Foundation of China (NO: 30371759, 90709018) and Natural Science Foundation of Shaanxi Province (2005C<sub>2</sub>50,GG06174).

## Open Access

This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

## References

1. Lutgens E, Daemen MJ (2002) Trends Cardiovasc Med 12:27–32
2. Bruemmer D, Riggers U, Holzmeiser J (2001) Am J Cardiol 87:21–27
3. Mach F, Schonbeck U, Sukhova GK, Atkinson E, Libby P (1998) Nature 394:200–203
4. Schonbeck U, Sukhova GK, Shimizu K (2000) Proc Natl Acad Sci USA 97:7458–7463
5. Schonbeck U, Libby P (2001) Circ Res 89:1092–1103
6. Lutgens E, Cleutjens KB, Heeneman S (2000) Proc Natl Acad Sci USA 97:7464–7469
7. Shim DH, Hee YK (2004) Arch Biochem Biophys 421:214–220
8. Liang GB, Zhang GP, Jin HM (2005) Chin J Pathophysiol 21:16–20
9. Zhang JT (ed) (1998) Modern experimental methods in pharmacology. Beijing Medical University and Beijing Xiehe Medical University Unite Press, Beijing, pp 999–1015
10. Lin R, Yang GD, Wang WR (2006) J Chin Med Mat 26:1315–1317